**Reviews for “Imaging of Stem Cells”**

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**DOI 10.3233/STJ-190003.** <https://content.iospress.com/articles/stemjournal/stj190003>.

(this Review article is an update of a Chapter of the same name on [www.StemBook.org](http://www.StemBook.org) that had gone through extensive review before)

**Reviewer 1** has selected to remain anonymous.

Originality, novelty and significance of results: Excellent

Technical Quality of Work: Good

Comprehensibility and Presentation of Paper: Good

What is the overall impression: Good

**Reviewer Recommendation Term:** Accepted pending minor revisions

 **Narrative (as sent to corresponding author):**

This manuscript is an update of a previously published chapter in StemBook. The authors described the different methodologies available to investigate and monitor transplanted stem cells in vivo. The pros and cons of different techniques and suitability for different experimental goals are well described. The addition of two new sections on "Translation to clinical application" and "Clinical application" is highly relevant. This manuscript would be valuable for stem cell researchers who intend to investigate the effects of stem cell transplant in vivo and for preclinical studies. There are some areas of the manuscript that could be expanded and improved for better flow as described below.

Major comments:
1. The quality of the figures in the current manuscript is low, the text in them are barely legible and some of them cannot be read at all due to low resolution. This may be due to conversion to PDF format although Figure 3 from the original published chapter also has low resolution. Please ensure that if the manuscript is accepted for publication that the figures are of adequate resolution.

2. In page 5 second paragraph, the sentence "The QDs have narrow emission spectrum and broad excitation spectrum and have demonstrated high resolution, long duration, high sensitivity and energy saving so they were applied to 4k display in 2013.(30)". What does energy saving mean in this context? It is unclear what the significance of "applied to 4k display" is. Does the authors mean that much higher resolution has been achieved compared to the past? In that case would it not be better to compare the different resolutions achievable now and in the past? How much of an improvement has been made?

3. In the same paragraph, please give a brief description of time-domain imaging as it becomes important later in the manuscript. In page 12 first paragraph, the authors wrote "Novel developments, such as time-domain imaging (79-81), are incorporating the time domain in the analysis, and have the potential to provide depth information of the fluorescent signal." is vague and does not give any information about the technique.

4. In page 13 the authors mentioned "Imaging in the red-shifted light spectrum results in higher signal-to-background ratio..." please expand a little bit on this reasoning. Is it because the background auto-fluorescence is in the yellow-green fluorescence range? Is it because longer wavelength (red and far-red) fluorescence have better tissue penetration and thus less signal loss due to tissue depth?

5. In Page 15 second paragraph, the authors mentioned that BLI is useful for studying interactions with the microenvironment but there was only one sentence in this section about microenvironment by Kutscha et al and it is very brief and lacking detail. Please expand.

6. In Page 22 last paragraph, the authors mentioned that direct labelling has been used in clinical applications with "varying success" and "within the spectrum that this methodology can answer" but did not elaborate. Please briefly expand on this section and give an example.

Minor comments:
1. It would be better to have all the figures labelled consistently. In figures 1, 3, 5 and 6 the authors labelled the parts of the figure A, B, C, etc. but for figures 2 and 4 the authors referred to the parts of the figures as left, middle and right. Labelling them A, B and C would be much easier to read and consistent with the other figures. Also, in Figure 2, right is referred to first in the text before middle, please re-order so that figures are referenced in chronological order.

2. In page 5 first paragraph, please reference the different parts of Figure 1, Figure 1A, 1B, 1C and 1D where appropriate. Please do the same for all figures in the manuscript.

3. The previous version of Figure 9 (Figure 6 in the published chapter) included references for the different techniques which I think is a nice addition and should also be included here with updated references.

4. There was a change in referencing style in some parts of the manuscript. For example, in page 14 the references are placed after the punctuations. Please make sure it is consistent.

5. There are some typographical errors in this manuscript that needs to be fixed:
Page 5 second paragraph "...fluorescent signal can be used...", please add "that".
Page 10 "...will results in", please remove the "s", just "result".
Page 17 second paragraph "...tissues like skin, esophagus; underscoring..." please make it "skin and esophagus".
Page 19 fourth paragraph "transferring" should be "transferrin".
Page 22 third paragraph "...used to identify were cells have been delivered" should be "where" not "were".
Page 23 first paragraph "reported gene" should be "reporter gene".
Same paragraph "FDA approved manor", should be "manner" not "manor".
Page 24 second paragraph "report gene" should be "reporter gene".

6. Figure legend for Figure 1, please include "panel D" in the figure legend for the description of MRI and the abbreviation should be "SPIO" not "SPO".

7. Figure legend for Figure 6. "reporter" not "report" and "Red fluorescent protein" not "Read fluorescent protein".

8. Page 21 second paragraph, please give some examples on what large animal models have been used.

**Reviewer 2** has selected to remain anonymous.

Originality, novelty and significance of results: Good

Technical Quality of Work: Excellent

Comprehensibility and Presentation of Paper: Excellent

What is the overall impression: Excellent

**Reviewer Recommendation Term:** Accepted pending minor revisions

 **Narrative (as sent to corresponding author):**

The authors have given a clear overview of the current methods to explore / track stem cells through imaging in various models. The use of figures and tables are clear. I don't think there is any thing extra required to significantly improve this manuscript.